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HEAVY METALS TOXICITY AND DETOXIFICATION STRATEGIES IN PLANTS

Monika Sood[1], Mamta Pujari[1] and Dhriti Kapoor[1]*
[1]Department of Botany, School of Bioengineering and Biosciences
Lovely Professional University, Phagwara- Punjab (India)-144411

Abstract

Contamination of environment by toxic metals is a serious ecological concern. In general terms, the elements which have density larger than or equal to 5 gcm^{-3} are referred to as heavy metals (HM). Though, HMs in traces acts as essential micronutrient in plant and animal communities, but in higher concentration they alter several cellular functions and by entering the food chain and undergo bio magnification at each trophic level and ultimately pose a risk to human health. HMs affect the plant by altering several metabolic processes at the cellular, tissue as well as molecular level. In present review paper we will summarize the adverse impacts of HMs induced toxicity in plants. In addition to this, we will also discuss about the various defensive mechanisms adopted by plants growing under heavy metals contaminated environment. Furthermore, the application of plants for toxic metal elimination i.e. phytoremediation is emerging as an effective strategy that could take care of HM-pollution. For its success, the understanding of mechanisms of HM stress effects and detoxification is necessary.

Keywords: Heavy metals; toxic effects; defensive mechanisms.

Introduction

In recent years, rapid changes in the environmental conditions have occurred. These abiotic activities insert deleterious effects on plant productivity and eventually yield (Schutzendubcl and polle, 2002; Kalra and Kumar, 2017; Kalubarme and Dubey, 2018; Swapnil *et al.*, 2018). Most of the environmental changes are associated with anthropogenic activities causing soil and air contamination include acid rain, soil erosion, salinity, enhanced UV B radiation, global warming, deforestation etc. (Kumar *et al.*, 2014; Mishra *et al.*, 2016; Singh *et al.*, 2014). Due to industrialization, ecosystems are exposed to a variety of pollutants with the risk of environmental pollution and human issues (Gerhardt *et al.*, 2009, Koo *et al.*, 2009; Dhami *et al.*, 2013). Ecological pollution by noxious metals has been observed to increase on alarming basis since the starting of global industrialization (Kabata-pendias and pendias, 1989). Heavy metals (HMS) are added to the environment through two main sources. Natural reservoirs of HMs are volcanoes and continental dust. In addition, HMS are deposited in the ecosystems as a consequence of various anthropogenic actions like, extracting and casting of metals, lustering, gaseous emission, energy & fuel creation, sewage & pesticide uses, community waste away production etc. (Denton, 2007). Following their release, heavy metals accumulate in plants and other living organisms and thus penetrate the food chain; ultimately, the human wellbeing is at hazard (Kumar *et al.*, 2018; Saggoo and Gupta, 2013; Galloway, *et al.*, 1982).

In contrast to the organic pollutants, which can undergo biodegradation as a result of activities of plants, soil microorganisms or abiotic factors, HMs are non-biodegradable. They are effortlessly accumulated in the soil and persist within the ecosystem for a prolonged period (Rajkumar *et al.*, 2005; Syed *et al.*, 2018; Kaur *et al.*, 2020). Elements possessing density more than 5 g cm^{-3} are designated as HMs (Gorhe and Paszkowski, 2006). Among the 90 elements occurring naturally in the environment, 53 are considered as heavy metals (Hasan *et al.*, 2009). Most of HMS are d and f transition elements that may form compounds and complexes with various non-metal donor atoms.

Depending upon the solubility under various physiological circumstances approximately, seventeen HMs are accessible and play essential role in growth and development of living organisms within innumerable bionetworks. A few HMs such as, Fe, Mn, MO, Zn, Ni, Cu, Cr serve as micronutrients and required in traces for cellular metabolisms. They play significant role by serving as cofactors for various enzymes, facilitating oxidation-reduction reactions and co-operating with nucleic acids as well as proteins (Kagi and Schaffer, 1988, Grotz *et al.*, 1998; Singh *et al.*, 2016; Kumar *et al.*, 2018). In contrast to this, HMs like, Hg, Ag, Cd, Pb and U are also available in the biosphere which seems to perform not even a single physiological function in plants. Rather, they are known to be noxious to both plants & microorganisms (Paasivirta, 2000). These metals penetrate to the living beings via the means of transport techniques exploited by crucial HMs (Paulsen and Saier, 1997, Thomine *et al.*, 2000, Rogers *et al.*, 2000). Additionally, there are also several metalloids that are toxic for plants including arsenic (Briat and Lebrun, 1999, Garnalero *et al.*, 2008).

Depending upon the reactivity with functional groups of biomolecules, HMs may also be classified into three types (Nieboer and Richardson, 1980).

Table 1 : Showing reactivity preference of HMs with different biomolecules

Classes	Heavy Metals	Reactivity Preference
A	Al^{3+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}	Oxygen(O >N>S)
B	Cu^{2+}, Hg^{2+}, Ag^{+}	Sulfur (S>N>O)
C (Border line)	Fe^{3+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}	Transitional affinity

Mechanism of heavy metal induced toxicity

The elevated strengths of almost every metal, comprising those crucial for advancement and metabolism, employ lethal consequences on metabolic traits of plants (Bajguz, 2000). The toxic threshold levels of metals in the tissues is termed as "stress point" can be well-defined as the concentration at which physiological property of a plant is irretrievably spoiled (Van Assche and Clijsters, 1990). Moreover, deadly properties of metals are also based upon the period and amount to which living beings are exposed (Nieboer and Richardson, 1980, Vallee and Ulmer, 1972). In view of the varying chemical properties of metals three diverse molecular methods of HM toxicity imposition can be differentiated.

- Generation of ROS by redox and Fenton reactions. These types of reaction are mainly exhibited by transition metals like, Fe and Cu.
- Hindering of important functional groups present in biomolecules owing to the affinities of heavy metals for thiols and other groups. These activities are mainly demonstrated by non-redox-reactive metals e.g. Cd.
- Dislocation of indispensable metal ions from biochemicals; these reactions are reported for different types of HMs (Schutzendubcl and polle, 2002).

Generation of Reactive Oxygen Species

Heavy metals are known to stimulate the production of ROS in cellular organelles such as peroxisomes and chloroplasts (Lopez *et al.*, 2007, Kapoor *et al.*, 2018; Singh *et al.*, 2020; Kapoor *et al.*, 2020). Heavy metals of organic implication can be allocated into two types namely i.e. redox active and redox inactive metals. HMs possessing redox potential less than the biomolecules are not able to take part in redox reactions of biological system. Furthermore, self-oxidation of redox metals like, Fe^{2+} or Cu^{2+} leads to consecutive reduction of molecular O_2 to H_2O_2 and yield the intermediates such as, O_2-•, $HO^{\cdot}$, and H_2O_2 which are theoretically more toxic as compared to molecular O_2 (Schutzendubcl and polle, 2002, Sharma and Dietz, 2009). Consequently, production of ROS may cause the undefined oxidation of biomolecules like, proteins and lipids or may leads to DNA damage. Accordingly, tissues bruised by oxidative damage commonly possess enhanced accumulation of carbonylated proteins, malondialdehyde and exhibit amplified generation of ethylene (Sharma and Dietz, 2009, Ames *et al.*, 1993).

Inactivation of cellular enzymes

Another strategy of HMs toxicity imposition is related with their strong affinity and bond formation with O, N and S atoms (Hasan *et al.*, 2009, Van Assche and Clijsters, 1990). This ability to form complex with these metals is because of free enthalpy of formation of products of HMs and ligands. Because of such features, heavy metals can deactivate the enzymes by combining with cysteine residues. Several enzymes contain metal ions that are significant for catalytic activity. The dislocation of a metal by other leads to either reduction in or lack of enzyme actions. For example, displacement of Mg^{2+} in Rubisco by divalent cations for instances, Co^{2+}, Ni^{2+} or Zn^{2+} resulted in disruption as well as loss of enzyme activity (Van Assche and Clijsters, 1986). Similarly, in plants like radish, in calmodulin, a shift of Ca^{2+} by Cd^{2+}, which serve an essential role during signaling pathway in plants result in lack of activity of phosphodiesterase enzymes which in turn activated by the binding of Ca^{2+} with calmodulin (Rivetta *et al.*, 1997). Similarly, an interruption in the photosynthesis process was also reported when in chlorophyll Mg is substituted by Pb (Neculita, 2005).

Owing to the above defined interferences, HMs affect the advancement and strength of plants by lowering cell wall mechanisms, cell lengthening as well as cellular tome (Macfarlane and Burchett, 2008). The membrane functioning in plant cells is influenced by HMs at the level of H+ ATPase activity ((Hasan *et al.*, 2009, Quariti *et al.*, 1997, Azevedo *et al.*, 2005). Disturbed water relationships (Kastori, *et al.*, 1992, Sharma and Dietz, 2006) and inhibited seed propagation (Al-Yemeni, and Al-Helal, 2003) by heavy metals have also been reported. Further, heavy metals not only decrease nodulation (Casella *et al.*, 1998) but also limits the development of microorganisms existing in the rhizosphere (Lorenzo *et al.*, 1994). HMS, for example, Pb reduce the translocation of water to foliar tissues by decreasing the number as well as vessels radii by partially blocking them with cellular debris and gums (Khan and Chaudhry, 2006)

Heavy metal decontamination and adaptive mechanisms in plants

Tolerance to HMs in plants refers to the capability to endure in a soil i.e. deadly to plants otherwise and is exposed by a communication among a genotype and its environmental setting (Hall, 2002). Plants possess a wide array of strategies at cellular as well as organ level that synergistically participate in detoxification, adaptation along with tolerance to HMs stress. These mechanisms comprise of a) cell wall attachment, b) alteration in permeability of ions, c) active exclusion, d) biotransformation, e) Outer as well as intracellular chelation and f) compartmentalization (Hall, 2002, Macfie and Welburn, 2000).

Extra and intracellular heavy metal chelation

Various kinds of exudates released by roots play several important roles towards the enhancement of chelation as well as uptake of a variety HMS from the soil (Marschner, 1995). For example, histidine and citrate exhibit the properties of Ni-chelation, gathered into the root exudates of non-hyperaccumulating plants and then aid in Ni cleansing strategy (Vyas, 2017; Vyas, 2019; Salt *et al.*, 2000). Similarly, Buckwheat is testified to discharge oxalic acid from the roots in retort to Al toxicity and collect nontoxic Al oxalates in the foliar tissue (MA *et al.*, 1997), therefore, detoxification can occur from both outside along with inside. HMs chelation inside the cytoplasm with ligands possessing high affinities towards them serve as an important and invaluable tolerance mechanism against metal-imposed stress in plants. Possible ligands comprise of amino acids, organic acid & two categories of peptides, i.e. phytochelatins and metallothioneins (Sharma and Dietz, 2006, Clemens, 2001, Rea, 1999).

Phytochelatins (PCs)

Studies involving cell culture established that the initiation of PCs accumulation in the occurrence of Cd accorded with an intermediate cut in level of GSH. Additionally, acquaintance of either cell culture or whole plants to GSH biosynthesis inhibitor i.e. buthionine sulfoxime (BSO), lead to amplified sensitivity to Cd with a consistent PCs biosynthesis inhibition. This might be inverted by increasing the concentration of GSH in growth medium. Further, these PC-HM complexes are moved towards the tonoplast, taken up by active transport and then sequestered inside the vacuole (Hall, 2002; Rea, 1999; Tommasini *et al*., 1998).

All steps of PC-dependent detoxification of heavy metals are supported by experimental evidence in circumstances of Cd and As. Other HMs like Ni, Al are, in fact, poor inducers of PCs (Steffens, *et al*., 1990). The application of PCs in heritable metal lenience has also been considered. For example, in case of zinc-sensitive & zinc tolerant Silene vulgaris, greater concentration of SH-GSH in the roots of zinc sensitive plants was evident when compared to that of the tolerant plants (Harmans *et al*., 1994). Parallel findings were attained in case of Cu in S. vulgaris (Schat and Kalff, 1992). These reports point that PCs might not elucidate the heritable Zn or Cu tolerance in S. vulgaris. It is further implied that PCs have the limitations with respect to the tolerance to the different HMs. Apparently; other means/mechanisms are available for the purpose.

Metallothioneins (MTs)

Metallothioneins are known to be involved in the management of inner concentrations of metals in between the scarce and lethal levels by enabling bond formation with toxic metals via closely set apart cysteine thiol groups. Structurally, metallothionens are gene programmed polypeptides which are generally categorized into two types. Class 1 MTs own cysteine residues that associate with mammalian renal MTs. Class 2 MTs also comprise cysteine like collections but these cannot be simply allied with class 1 MTS (Hall, 2002, de Miranda *et al*.,1989). In plants, metals like Cu, Zn, Cd have been reported to be bound by MTS ((Hall, 2002, Tomsett, Thurman, 1988). Though, MTS can be persuaded by Cu induced toxicity and there is also indication for their part in HMs tolerance in fungi and animals (Hamer, 1986), the function of MTs remain to be recognized ((Hall, 2002).

Organic acid and amino acid involvement in HM detoxification

Histidine has been shown to be involved both in the means of Ni tolerance and in the extraordinary degree of Ni transportation in the xylem (Kramer, 1996; Chauhan *et al*., 2017; Farooq and Sehgal, 2019a, 2019b)) required for hyperaccumulation in the shoots of i. Plants exposed to toxic HM concentrations have been reported to Produce free proline in high amounts (Sharma and Dietz, 2009, Bassi and sharma, 1993). Several functions for proline are possible in the light of available literature. These include 1) HM dependent osmotolerance as HMS are known to deteriorate plant water relations. 2) Possible HM-Pro complexation; this was evident in the in vitro protection of enzymes against HM poisoning (Sharma *et al*., 1998) 3) Proline being a component of cellular antioxidation defense; free radical scavenging features of proline have been demonstrated in certain in vitro assays e.g. graft co-polymerization assays involving the free radical facilitated grafting of a monomer (polyacrylamide) onto the cellulose backbone (Kaul, *et al*., 2008; Nankar *et al*., 2017; Mishra, 2019a, 2019b).

Antioxidative Defense Mechanisms

Since oxidative stress is tangled in HM toxicity incidences, the role of antioxidants in reduction of HM toxicity is reasonable. The antioxidative defense mechanisms include both non-enzymatic as well as enzymatic participants (Prabhakar *et al*., 2013; 2014; 2020; Sharma *et al*., 2019). Major antioxidant metabolites are ascorbate, reduced glutathione, α-tocopherol carotenoids, polyamines & flavonoids. The enzymatic antioxidants include catalase, ascorbate peroxidase, dehydroascorbate reductase, glutathione reductase, SOD (Buchanan *et al*., 2000; Singh *et al*., 2016; Singh *et al*., 2019). Evidence from experiments also involving appropriate mutants support these facts. HM tolerance, among other features, could be ascribed to the activity to efficiently manage cellular ROS through antioxidative defense (Sharma and Dietz, 2009; Sudhakar et., 2015).

Conclusion

The heavy metals added to the environment either through natural sources or as a result of anthropogenic activities, interfere with its quality. HMs accumulate in the plants affecting their productivity and yield. By entering the food chain, metals cause several serious health problems in human beings as well as other organisms. Although, in recent years, considerable progress towards the understanding of plant reactions to HMs stress has been achieved, the HM detoxification and tolerance mechanisms are not fully understood. For instance, the pathways responsible for perception and signaling of HM stress need to be characterized. Possibility of contribution of signaling molecules like, salicylic acid (Metwally *et al*., 2003), nitric oxide (Beligini and Lamattina, 2000; Neill *et al*., 2003; Sharma, 2008) abscisic acid (Sharma and Kumar, 2002) in the process is likely. In fact, the part of phytohormones, if any, in HM toxicity alleviation has not yet been fully explored.

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